Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Effect of physico-chemical characteristics of natural deep eutectic solvents on extraction of bioactives from fresh kinnow mandarin flavedo

Taru Negi
tarunegi12@gmail.com
Govind Ballabh Pant University of Agriculture and Technology
Anil Kumar
onlineclasses.drak@gmail.com
Govind Ballabh Pant University of Agriculture and Technology
Satish Kumar Sharma
Govind Ballabh Pant University of Agriculture and Technology
Anuradha Dutta
Govind Ballabh Pant University of Agriculture and Technology
Navin Chand Shahi
Govind Ballabh Pant University of Agriculture and Technology
Om Prakash
Govind Ballabh Pant University of Agriculture and Technology
Ashutosh Dubey
Govind Ballabh Pant University of Agriculture and Technology
Prarabdh C. Badgujar
NIFTEM-K

Research Article

Keywords: NADES, physicochemical, ultrasonication assisted extraction, bioactive compounds, cytotoxicity

Posted Date: October 27th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11484227/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: The authors declare no competing interests.

Effect of physico-chemical characteristics of natural deep eutectic solvents on extraction of bioactives from fresh kinnow mandarin flavedo

Taru Negi[a,1], Anil Kumar[b*], Satish Kumar Sharma[b], Anuradha Dutta[c] , Navin Chand Shahi[d]
Om Prakash[e], Ashutosh Dubey[f], Prarabdh C. Badgujar[g]

[a] Department of Food Science and Technology, College of Agriculture, Pantnagar, Uttarakhand, India
[b] Department of Food Science and Technology, College of Agriculture, Pantnagar, Uttarakhand, India
[c] Department of Foods & Nutrition, College of Community Sciences, Pantnagar, Uttarakhand, India
[d] Department of PHPFE, College of Technology, Pantnagar, Uttarakhand, India
[e] Department of Chemistry, College of Basic Sciences, Pantnagar, Uttarakhand, India
[f] Department of Bio-chemistry, College of Basic Sciences, Pantnagar, Uttarakhand, India
[g] Department of Food Science and Technology, NIFTEM, Kundli, Sonipat , Haryana, India

[1] Present address: Department of Post-Harvest Management, College of Horticulture, Veer Chandra Singh Garhwali Uttarakhand University of Horticulture and Forestry (VCSG UUHF), Bharsar, Uttarakhand, India.

[*]Corresponding author: Dr. Anil Kumar, email address- onlineclasses.drak@gmail.com

Abstract

Citrus peel waste is known for its rich treasure of bioactive compounds that has multiple health benefits. In this study, sixteen natural deep eutectic solvents (NADESs) were prepared, from different combinations of choline chloride with hydrogen bond donors such as acid, alcohol and sugar. Physicochemical analysis of these solvents such as pH, viscosity, density, and polarity were examined. Results revealed that extract obtained by using some NADESs as an extraction solvent showed higher total phenol content, total flavonoid content and antioxidant capacity than conventional solvent (80 % methanol). The ultrasonication assisted extraction (UAE) proved to be effective technique for extraction of bioactive compounds in

less time (i.e. 30 min) at 50 ℃ temperature as compared to reflux (2 h at 80 ℃) method. Also, cytotoxicity of best NADESs combinations were examined in order to ensure the safety consumption of it when used as solvent for extraction of bioactive compounds.

Keywords: NADES, physicochemical, ultrasonication assisted extraction, bioactive compounds, cytotoxicity

Introduction

Fruits wastes are the storehouse of health beneficial compounds but it goes in vain due to shortage of knowledge, space, funds and technology by the food processing industries for their further utilization [1]. It is well known that peels of some fruit contain high amount of bioactive compounds than its edible part and one such fruit is citrus. In 2025-2026, global fresh orange production is projected to reach 45.92 million metric tons, while fresh tangerine/mandarin, lemon/lime, and grapefruit production are estimated at 38.40, 10.06, and 7.00 million metric tons, respectively [2]. The flavedo (colored part) extracts of citrus peel represent a significant source of phenolic antioxidants having prophylactic properties for the development of functional foods [3]. These valuable and functional components can be recovered by employing different extraction methods.

Ultrasonication assisted extraction (UAE) categorized as a green technology stands out for its eco-benign, simple, cost-effective, and time-saving nature. This method also excels in preserving both heat-sensitive and unstable compounds over traditional methods like reflux, maceration, and soxhlet. The application of ultrasound induces acoustic cavitation, leading to the formation, expansion, and collapse of bubbles. These cavitation bubbles, concentrated near the solid surface, rupture with greater intensity, facilitating the rupture of cell walls and expediting the release of desirable molecules into the solvent medium [4]. The industry is also looking for environment friendly solvents and sustainable extraction techniques, following the principles of green chemistry.

Conventional organic solvents, such as hexane and others are used traditionally for extraction which are toxic, flammable, explosive and poorly degraded. Extract obtained from these solvents contain residual portions of toxic chemicals which is physiologically and environmentally harmful. Thus, the drawbacks of extractions using organic solvents can be overcome by using NADESs [5] . NADESs, composed of hydrogen bond acceptors (HBAs) like choline chloride (ChCl) and hydrogen bond donors (HBDs) such as polyalcohol, sugar etc. which are effortlessly prepared by heating and mixing.

Extraction of phenolics and terpenoids were carried out by Vo et al. [6] from passion fruit peel by coupling NADESs with UAE and found lactic acid:glycerine and citric acid:glucose NADESs higher in extracting phenolics and terpenoids, respectively. Cvjetko Bubalo et al. [7] found ChCl:oxalic acid (molar ratio 1:1) DES with 25 % water content best for extracting phenolics from grape skin. Viñas-Ospino et al. [8] found highest extraction of carotenoids from orange peel when octanoic:L-proline hydrophobic deep eutectic solvent (DES) coupled with UAE. M. Xu et al. [9] observed ternary ChCl:levulinic acid:N-methyl urea based DES best for the extraction of total flavonoid (18.75 mg/g polymethoxy flavonoid and 47.07 mg/g glycosides of flavonoid) from citrus peel. However, N-methyl urea component used in preparation of DES has been classified as probable human carcinogen by U.S. Environmental Protection Agency [10].

While going through the literature, there was no work citing selection of NADESs, based on their physicochemical characteristics for extraction of bioactives from kinnow mandarin flavedo. Hence, in the present study, sixteen (16) different combinations of NADESs (acid, alcohol, sugar based) at different molar ratios were tried in order to check their flow for efficient extraction of bioactive compounds from kinnow citrus peel. Also, cytotoxicity of NADESs (without extract) were also tested so that it can be safely used for human consumption.

Materials and methods

Raw material and chemicals

Kinnow citrus fruit were purchased from local market. Folin-ciocalteu, quercetin, 2, 2-Diphenyl-1-picryhydrazyl (DPPH), 2,2-azinobis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) and 2,4,6-tripyridyl-s-triazine (TPTZ), ferric chloride, gallic acid, quercetin, butylated hydroxytoluene (BHT) were purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Mice for cytotoxicity experiment was sourced from National Institute of Biologicals, Noida, India.

Preparation of NADESs

To prepare NADESs solution for screening purpose, component 1 was mixed with component 2 in the ratio mentioned in Table 1. The combinations were heated (without addition of water) on hotplate at 80 ºC and stirred till it become completely liquefied to a clear solution [11].

Table 1 Combination of NADESs selected for study

Sr.	Component 1	Component 2	Molar ratios
1.	ChCl	Glucose (Glu)	1:1
2.	ChCl	Glucose (Glu)	2:1
3.	ChCl	Sucrose (Suc)	1:1
4.	ChCl	Oxalic acid (Ox)	1:1
5.	ChCl	Malic acid (MA)	1:1
6.	ChCl	Lactic acid (LA)	1:1
7.	ChCl	Lactic acid (LA)	1:2
8.	ChCl	Lactic acid (LA)	2:1
9.	ChCl	Levulinic acid (Lev)	1:1
10.	ChCl	Glycerol (Gly)	1:1
11.	ChCl	1, 3 butanediol (1,3 BD)	1:1
12.	ChCl	1, 4 butanediol (1,4 BD)	1:1
13.	ChCl	1,2 Propanediol (1,2 PD)	1:1
14.	CA	Glucose (Glu)	1:1

15.	CA	Glucose (Glu)	1:2
16.	CA	Glucose (Glu)	2:1

ChCl - Choline Chloride, CA - Citric Acid

Visual appearance of prepared NADESs was observed. Further, 10-40 % of water was added along with component 1 and 2 followed by heating the mixture only in those NADESs combinations that showed solidification or partial solid/partial liquid or crystalline behavior. For final study, 30 % distilled water (component 1 (g) + component 2 (g) × 30/100) was added along with component 1 and 2 followed by heating the mixture as described above.

Preparation of fresh citrus flavedo and its extract

Flavedo part (thickness 1.4 mm) of kinnow citrus fruits were peeled and vacuum packed followed by storage in deep freezer (-18 °C) till further use. With slight modification in the method of [12], for preparation of extract, fresh kinnow citrus flavedo (1 gm) was weighed, crushed in pestle mortar followed by addition of 20 mL of different NADESs. Whole solution was poured in a beaker and UAE (LABMAN Probe Sonicator PRO650, India) with 6 mm probe was performed at given conditions i.e. solid: liquid (1:20 g/mL), water content (30 %), temperature (50 ºC), time (30 min), power (200 W) and pulse working mode (ON for 3 sec and OFF for 3 sec). Solution was centrifuged (R-23 REMI Research Centrifuge, Mumbai, India) at 4500 RPM for 20 min followed by collection of supernatants. All extracts were made up to uniform volume (25 mL) with respective solvents and stored at 4 ºC in amber glass vials till further analysis. For control, 80% methanol was used as a solvent and refluxed at 80 ºC for 2 h.

Analytical Methods

pH

The pH values were determined by using Hanna Digital pH meter (HI 5221, Hanna Instruments, India) at 28 ºC [13]. The instrument was calibrated with pH 4.0, 7.0 and 9.2 before

124 measuring pH of the solvents and each time, electrode was rinsed with distilled water before
125 dipping in various solvents.

126 ***Viscosity***

127 Viscosity of solvents were measured according to Nazir et al. [14] by Ostwald's
128 viscometer at 22℃ by using Eq. (1).

129 $$\eta_2 = \frac{\rho_2 t_2}{\rho_1 t_1} \times \eta_2 \quad (1)$$

130 where η_2 = viscosity of solvent (cP), ρ_2= density of solvent (g/mL), t_2= meantime of flow of
131 solvent from A to B (sec), ρ_1= density of water (g/ml), t_1 = meantime of flow of water from A
132 to B (sec), η_1 = viscosity of water (cP)

133

134 ***Density***

135 Density of all the solvents were measured according to Freitas et al. [15] by weighing
136 25 mL of solvent in pycnometer (25 mL) at 16 ℃ for NADESs combinations and 80 %
137 methanol. It was calculated by Eq. (2). s

138 $$\text{Density} = \frac{\text{mass of different NADES solvent}}{\text{volume (25 mL).}} \quad (2)$$

139 ***Polarity***

140 Polarity testing was done by slight modification of Gómez-Urios et al. [16] method.
141 Polarity was measured in the form of molar transition energy (E_{NR}) by using a solvato-
142 chromatic probe i.e. Nile red. Firstly, 1000 µl of solvent was pipette in cuvette followed by
143 addition of 50 µl Nile red solution (10 mg/10 mL of ethanol) and then, absorbance was

144 measured from 400-700 nm. The absorption spectra of dyes (λ_{max}) were determined with a
145 UV/Vis spectrophotometer and calculated by Eq. (3).

146 $$E_{NR}\,(\text{kcal moL}^{-1}) = \frac{hcN_A}{\lambda_{max}} = \frac{28{,}591}{\lambda_{max}} \quad (3)$$

147 where, Planck constant (h) is 6.63×10^{-34} J/s, velocity of light (c) is 2.997×10^{8} m/sec,
148 Avogadro constant (N_A) is 6.022×10^{23} mol^{-1} and Lambda max (λ_{max}) is wavelength at the
149 maximum absorbance

150 ***Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)***

151 TPC of kinnow flavedo extract was determined by using Folin–Ciocalteu assay
152 described by Boli et al. [17] with slight modifications. All experiments were performed in
153 triplicate. For each determination, 300 µl of extract was combined with 7700 µl of distilled
154 water and 500 µl of Folin-Ciocalteu reagent, and the mixture was vortexed, Then, 1500 µl of
155 sodium carbonate (20 % w/v) was added followed by addition of 1500 µl sodium hydroxide (4
156 % w/v, in order to avoid precipitation) and mixed thoroughly. Mixture was kept in a water bath
157 at 40°C for 30 min or until the precipitate had disappeared. The mixture was cooled, and
158 absorbance was measured at 765 nm in a UV-Vis spectrophotometer (Eppendorf Bio
159 spectrometer basic, Germany). The TPC was expressed in mg gallic acid equivalent per gram
160 of peel (mg GAE/g dry weight basis, dw).

161 TFC was measured using method of Che Zain et al. [18] with slight modifications.
162 Extract (80 µl) was taken in a test tube and 420 µl of 95% ethanol was added to make up the
163 volume to 500 µl. Then, 25 µl aluminum chloride (10%), 25 µl sodium acetate solution (1 M)
164 and 700 µL distilled water were added and incubated (25°C for 40 min) followed by final
165 measurement of absorbance at 415 nm. The total flavonoid content (mg QCE/g dw) was
166 expressed in mg quercetin equivalents per gram of peel.

Antioxidant activity assays and Antioxidant potency composite index (APCI)

Antioxidant activity was measured by using DPPH, ABTS and FRAP assay. For the DPPH assay, 130 µL of extract was added to 5 mL of 0.004% DPPH solution prepared in methanol. The reaction mixture was maintained at ambient temperature for 30 min, after which absorbance was recorded at 517 nm against methanol as the blank [19]. ABTS radical scavenging activity was determined following the procedure described by Belwal et al. [20] and absorbance was measured at 734 nm using a UV–visible spectrophotometer. Ferric reducing antioxidant power (FRAP) was assessed according to Singanusong et al. [21]. The reaction mixture was incubated in the dark at 25 ± 2 °C for 30 min, and absorbance was subsequently measured at 593 nm. Results were expressed in mg BHT equivalent per gram dry weight of citrus peel (mg BHTE/g dw).

Antioxidant Potency Composite Index (APCI) was calculated according to method of Chen et al. [22] by Eq. (4).

$$\text{APCI} = \frac{\text{measured value of assay (sample score)}}{\text{maximum value of assay (best score)}} \times 100 \quad (4)$$

Further, average of index score of all three assays (DPPH, ABTS, FRAP) was calculated for each NADES combination and named as overall antioxidant potency composite index to give more comprehensive idea of antioxidant activity of extracts.

Cytotoxicity

Cytotoxicity was evaluated using the MTT assay in mouse splenic lymphocytes according to [23]. Cells were treated with different concentrations of NADES for 24 h, followed by MTT incubation and absorbance measurement at 570 nm. The % viability of the cells was calculated using the following formula

$$\text{Cell Viability (\%)} = \frac{AT}{AC} \times 100$$

Where, AT - absorbance of treated cells, and AC - absorbance of untreated cells

Statistical analysis

Data is expressed as the means ± standard deviation of three replicates. One - way ANOVA with post-hoc Tukey's HSD multiple comparison test was performed by SPSS 16.0 software, whereas graphs were prepared using GraphPad Prism 8.0.1 version software.

Results and Discussion

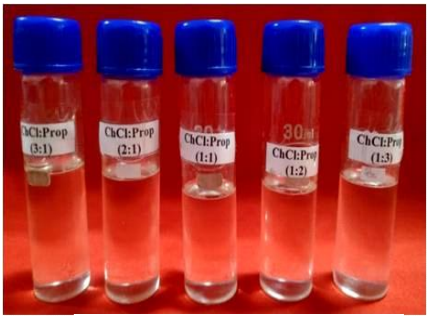
Screening of NADESs

Screening of NADESs was done keeping in mind the ideal characteristics of solvents required for extraction i.e. flowability, viscosity and transparency/clarity. Sugar based NADESs combinations (ChCl:Glu, ChCl:Suc and CA:Glu) in all molar ratios (without addition of water) were found to be either solid or partially solid and partially liquid after 24 and 48 h (Fig. S1). These pair of NADESs found highly viscous even after adding 40 % of distilled water, which was consequently difficult to handle during experimentation. Acid based NADESs (without addition of water), except ChCl:Ox and ChCl:LeA were clear, transparent and flowable after 48 h (Fig. S2). ChCl:Ox and ChCl:LeA NADESs showed thread like structure of crystals after 24 and 48 h, respectively without water addition (Fig. S3). Alcohol based NADESs combinations (ChCl: Gly, ChCl: 1,3 BD, ChCl: 1,4 BD and ChCl: 1,2 PD) were found less viscous than acid based (ChCl: LA, ChCl: LeA and ChCl:MA) and sugar based NADESs (ChCl: Glu, ChCl: Suc and CA: Glu). Therefore, keeping all above points in mind and based on preliminary trial, ChCl: 1,2 PD, ChCl: LA, ChCl: MA, ChCl: Gly and ChCl: 1,3 BD were selected for final trials because oxalic acid and 1, 4 BD chemicals were not listed in GRAS list. Also, ChCl: Glu, ChCl: Suc, CA: Glu (sugar based NADESs) were rejected as they

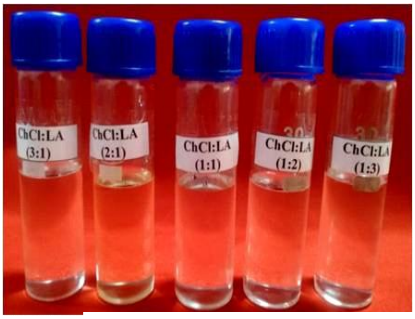
were very highly viscous in nature even after addition of distilled water whereas ChCl: LeA based NADES was found less in antioxidant potency composite index (data not shown).

Physicochemical characteristics of NADESs

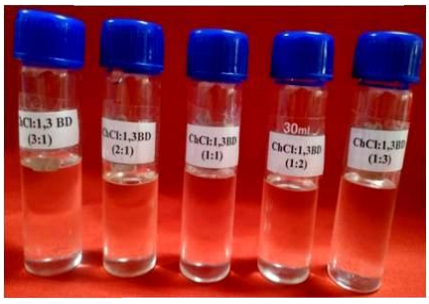
The extraction performance of NADESs is governed by both the structural features of the bioactive compounds and solvent characteristics, particularly polarity, viscosity, density, and pH [23]. Therefore, pH, density, viscosity and polarity of different ChCl based NADESs (water content 30 %) at respective temperature were investigated in the first part of this work (Table 2). Mostly all prepared ChCl based NADESs were found colorless in appearance except malic acid which was light yellowish. Furthermore, ChCl:MA NADESs with molar ratios 1:2 and 1:3 was noted unstable as white precipitate settled down at the bottom of the beaker and therefore, were not taken for the further study and eliminated (Fig. 1).



a) ChCl:1,2 Prop



b) ChCl:LA



c) ChCl:1,3 BD



d) ChCl:Gly

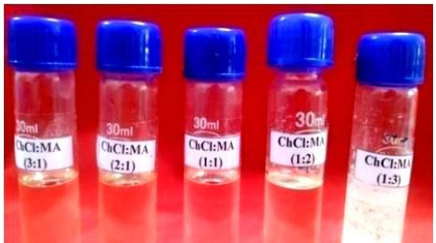




Fig. 1 Physical appearance of NADESs at various molar ratios (3:1, 2:1, 1:1, 1:2, 1:3)

Table 2 Physicochemical Properties of different NADESs used in study[#]

NADES Combination	Molar Ratio	Appearance of NADES	pH	Viscosity (cP)	Density (g/mL)	Polarity (kcal/mol)
	3:1	Colourless	4.63 ± 0.16	13.96 ± 0.36	1.12 ± 0.05	49.83 ± 0.10
	2:1	Colourless	4.73 ± 0.14	11.60 ± 0.54	1.12 ± 0.04	50.13 ± 0.10
ChCl:1,2 PD	1:1	Colourless	4.02 ± 0.04	11.39 ± 0.11	1.11 ± 0.01	49.69 ± 0.09
	1:2	Colourless	4.37 ± 0.09	7.60 ± 0.31	1.10 ± 0.03	49.95 ± 0.10
	1:3	Colourless	5.00 ± 0.21	6.90 ± 0.20	1.10 ± 0.03	49.98 ± 0.05
	3:1	Colourless	4.12 ± 0.02	8.02 ± 0.13	1.15 ± 0.02	49.81 ± 0.06
	2:1	Colourless	4.62 ± 0.02	9.17 ± 0.22	1.16 ± 0.05	49.53 ± 0.09
ChCl:Gly	1:1	Colourless	3.58 ± 0.16	7.95 ± 0.13	1.17 ± 0.02	49.38 ± 0.01
	1:2	Colourless	4.43 ± 0.13	8.49 ± 0.57	1.18 ± 0.03	49.06 ± 0.09
	1:3	Colourless	4.81 ± 0.01	9.31 ± 0.32	1.19 ± 0.03	49.01 ± 0.02
	3:1	Colourless	4.06 ± 0.17	11.83 ± 0.32	1.12 ± 0.01	49.92 ± 0.05
	2:1	Colourless	3.84 ± 0.07	11.33 ± 0.37	1.11 ± 0.03	49.61 ± 0.10
ChCl:1, 3 BD	1:1	Colourless	3.45 ± 0.12	12.79 ± 0.21	1.10 ± 0.04	50.06 ± 0.01
	1:2	Colourless	4.19 ± 0.02	11.15 ± 0.15	1.08 ± 0.09	49.80 ± 0.06
	1:3	Colourless	4.69 ± 0.06	10.52 ± 0.39	1.07 ± 0.03	49.71 ± 0.06
	3:1	Light yellowish	1.48 ± 0.05	17.65 ± 0.45	1.19 ± 0.01	48.30 ± 0.05
ChCl:MA	2:1	Light yellowish	1.09 ± 0.03	17.92 ± 0.26	1.20 ± 0.01	48.20 ± 0.02
	1:1	Light yellowish	0.03 ± 0.06	19.37 ± 0.27	1.24 ± 0.06	48.03 ± 0.06
	3:1	Colourless	2.09 ± 0.07	9.33 ± 0.15	1.14 ± 0.05	48.90 ± 0.09
	2:1	Colourless	1.70 ± 0.03	8.24 ± 0.12	1.15 ± 0.02	48.67 ± 0.10
ChCl:LA	1:1	Colourless	1.33 ± 0.01	8.24 ± 0.25	1.16±0.03	48.30 ± 0.01
	1:2	Colourless	1.03±0.01	6.14 ± 0.12	1.17 ± 0.04	48.17 ± 0.11
	1:3	Colourless	0.89 ± 0.02	5.34 ± 0.05	1.17 ± 0.03	48.04 ± 0.01
80 % Methanol		Colourless	7.45 ± 0.10	0.80 ± 0.52	0.82 ± 0.08	48.96 ± 0.13
	C.D.		0.17	0.48	0.04	0.11

S.E. (m)	0.06	0.17	0.02	0.04
S.E. (d)	0.09	0.24	0.02	0.06
C.V.	3.06	2.85	2.11	0.14

diluted with 30% distilled water ,all values are mean ± standard deviation of three replicates

It is well known that the various kinds of hydrogen bond donor (HBD) are known to have a significant impact on the acidity or pH of NADESs [24]. In this study, the pH range of all the NADESs combinations were found in acidic side ranging from 0.03 to 5.00. Lowest pH was found in combination ChCl:MA NADES at molar ratio of 1:1 (pH 0.03) whereas highest pH 5.00 was noted in ChCl:1,2 PD NADES at molar ratio of 1:3. On the other hand, methanol (80 %) had pH of 7.45 which was near to neutral range. Lower values of pH for ChCl:MA and ChCl:LA based NADESs as compared to others might be apparently because of the existence of acidic chemicals as HBD which is ultimately responsible for acidic nature of eutectic mixtures [26].

Highly viscous solvents hinder in mass transfer rate of the target solute consequently resulting in poor extraction. This problem can be solved by adding water during preparation of different NADESs to reduce its viscosity and increasing the extraction efficiency of bioactive compounds. In this study, 30 % of water was added in all prepared NADES as further addition of excessive water beyond 30 % results in weak interaction between NADES and target compounds, adversely affecting extraction process [27]. Viscosity of all the prepared NADESs was found higher than that of 80 % methanol (0.80 cP). Higher viscosity values i.e., 17.65 cP (molar ratio 3:1), 17.92 cP (molar ratio 2:1) and 19.37 cP (molar ratio 1:1) were observed in ChCl:MA based NADESs whereas lower values were found in ChCl:LA based NADESs at molar ratio of 1:2 (6.14 Cp) and 1:3 (5.34 cP). [28] also found high viscosity of ChCl:MA (446 cP) at molar ratio 1:1, followed by ChCl:Gly (113 cP) and ChCl:1,2 PD (25 cP) at molar ratio of 1:2 in their study. Generally, the viscosity is higher if one of the NADES components has more hydroxyl groups that act as hydrogen bond donors (HBD), thus creating more hydrogen

bond formation resulting in more viscous eutectic mixture. This might be the reason behind the high viscosity of ChCl:MA based NADES than other combinations as MA has three hydroxyl groups whereas other HBD (1,2 PD, LA and 1,3 BD) carry only two hydroxyl group except glycerol which also have three hydroxyl groups. Even though glycerol has three hydroxyl groups (OH in cis form), its viscosity was found less as compared to malic acid (OH in transform) due to positioning of hydroxyl group present in the compound because viscosity of trans-compound is greater than that of the corresponding cis isomer [29].

Density is an important physicochemical parameter that influences solvent diffusivity and its capacity to mix uniformly with other liquids. It plays an important role due to its effect on the design and process operation as higher density of DESs during extraction process will require higher power consumption during agitation or mixing of contents using agitators or magnetic stirrers and, during conveyance through pump from one place to another [30].Density of all tested NADESs were found in the range of 1.07 - 1.24 g/mL and observed higher than water (1.01 g/mL) and ethanol (0.82 g/mL). Highest density was found in ChCl: MA based NADES i.e. 1.24 g/mL at molar ratio 1:1 whereas lowest was noted in ChCl: 1,3 BD (1.07 g/mL) at molar ratio 1:3. This might be due to high molecular weight (Mw) compounds which possess higher density than low Mw compounds. For example, in this case, MA as HBD has higher Mw (134.08 g/mol) than other HBD (LA 90.08 g/mol, Gly 92.09, 1,2 PD 76.10 g/mol and 1,3 BD 90.12 g/mol) that resulted in higher density of ChCl: MA NADES. Alcohol based ChCl: 1,2 PD and ChCl:1,3 BD NADES had almost same density value range. The results of this study are in agreement with previous findings of Che Zain et al. [18] and Dai et al. (31)who noted similar density values of alcohol based 1, 2 PD and 1,4 BD NADESs.

Polarity is one of the main attributes of NADESs that plays a major role in extraction of higher content of bioactive compounds that can be evaluated by molar transition energy (E_{NR}). Less polar NADES gave pinkish colour whereas high polar NADES gave bluish colour

with Nile red dye (Fig. 2). High value of E_{NR} means low polarity whereas low E_{NR} values mean high polarity of NADESs [32]. E_{NR} value of different NADESs taken for study ranged from 48.03 to 50.13 kcal/mol (Table 2). ChCl:MA (1:1) was found most polar (48.03 kcal/mol) and ChCl:1,2 PD (2:1) was noted least polar (50.13 kcal/mol) in nature. Overall, organic acid-based NADESs (ChCl:LA and ChCl:MA) were observed to be having high polarity (less E_{NR} value) than alcohol based NADESs (ChCl:1,2 PD, ChCl:Gly and ChCl:1,3 BD).

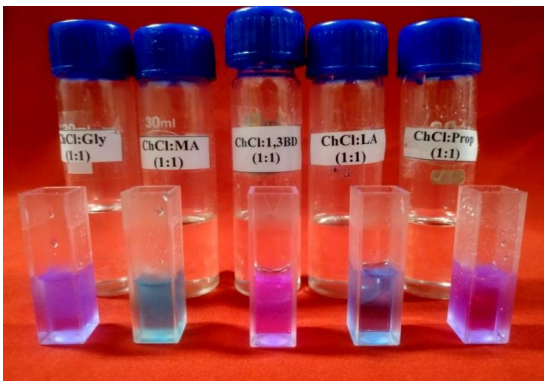


Fig.2 Polarity of NADESs at molar ratio 1:1 (pinkish – less polar, bluish – more polar)

Dai et al. [31] also found the high polarity of organic acid-based NADES (44.81 Kcal/mol) whereas both sugars as well as polyalcohol based NADESs were noted least polar. Similarly, Gómez-Urios et al. [16] noted more apolar nature of ChCl:Gly with polarity of 49.00 kcal/mol than ChCl:MA (47.85 kcal/mol). Due to their intrinsic high polarity, hydrophilic NADES typically have a high solubilization capacity for polar metabolites, but they can also be used with some non-water-soluble metabolites. Polarity of 80% methanol was found 48.96 kcal/mol which was somewhat near to the polarity of acid based NADESs.

Evaluation of NADES extraction efficiency

Extraction efficiency of different NADESs was assessed by considering the TPC, TFC and antioxidant capacity of the kinnow flavedo extract. During estimation of TPC, extract obtained from NADESs gave yellowish turbid solution after addition of FC reagent which precipitated out after sometimes (Fig. 3). It might be because FC assay works in alkaline medium (pH - 10) by transferring electrons from phenolic compounds present in sample to the phosphomolybdic/ phosphotungstic acid complexes (present in Folin–ciocalteu reagent) which form blue complexes [33]. To make the medium alkaline for successful reaction process, sodium hydroxide (4 % w/v) was added which resulted in prevention of precipitate formation and consequently, giving blue colour.

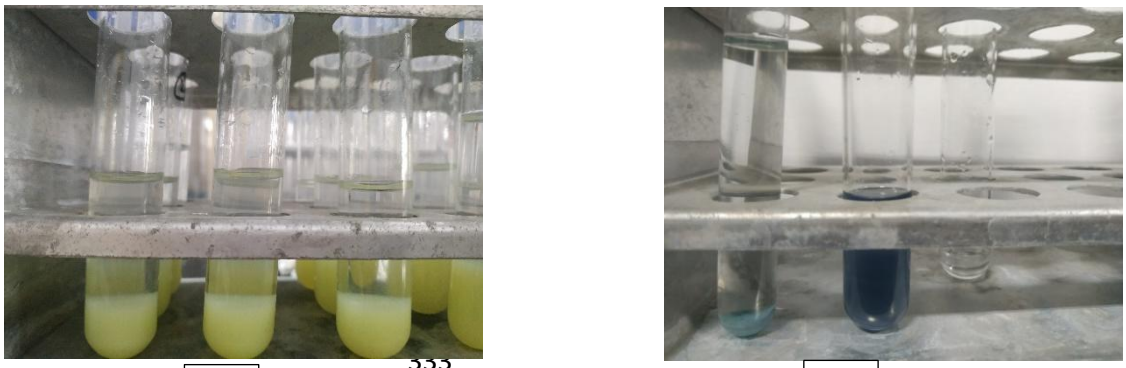


Fig. 3 Precipitation during estimation of Total Phenolic Content (TPC) in extract obtained from NADES (a) after addition of FC reagent (b) after addition of sodium carbonate (first from left) and sodium hydroxide (second from left)

Among all NADES and with their respective molar ratios, highest TPC (32.38 mg GAE/g) was found in the extract obtained by ChCl:MA based NADES at molar ratio 1:1 (Fig. 4A). Gómez-Urios et al. [5] also noted highest TPC in ChCl:MA NADES (i.e. 10.53 mg GAE/g) in *Citrus sinensis* peel which is less than our findings which might be due to employing different extraction condition or difference in raw material (citrus peel) on the basis of geographical origin, plant part and harvesting. Conventional solvent used in this study i.e. 80 % methanol showed TPC of 15.99 ± 0.31 mg GAE/g dw. Choline has a positively charged quaternary amine group, which could promote electrostatic interactions with the negatively

charged/polarized polyphenols and, in this way, enhanced their extraction (García-Roldán et al. 2023). Overall, approximately two-fold higher TPC was noted in ChCl:MA based extract (molar ratio 1:1) than conventional solvent (80 % methanol) which might be due to the lack of extractability of partially ionized compounds by methanol, where electrostatic interactions might have significantly contribute to their extraction [35]

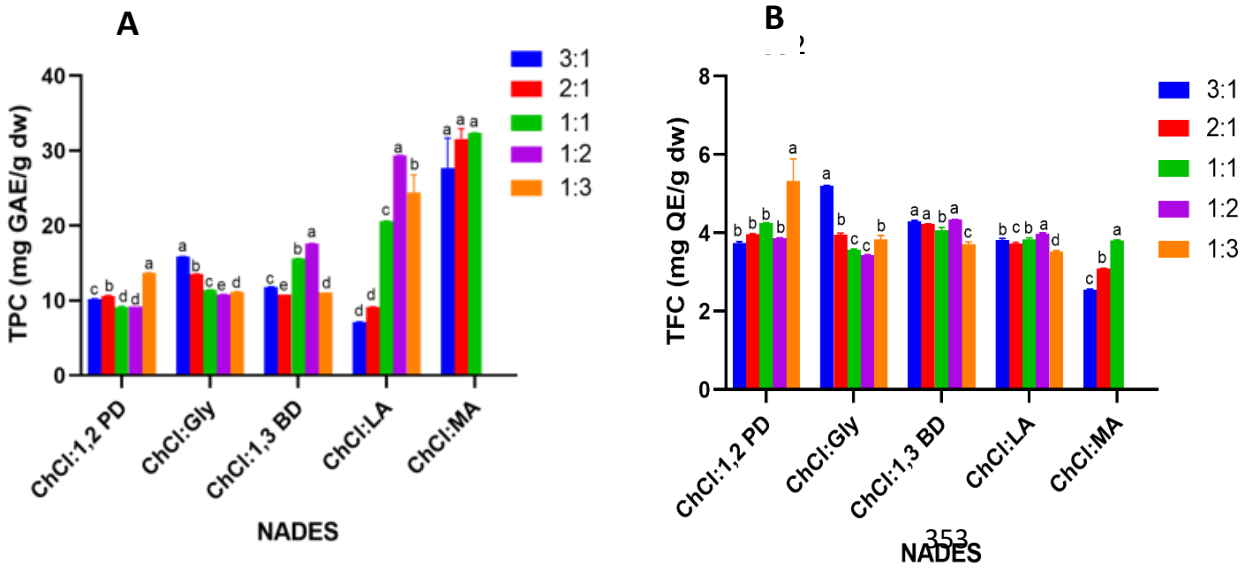


Fig. 4 Extraction efficiency of five NADES at different molar ratios (A) Total phenolic content (TPC) (B) Total flavonoid content (TFC)

Flavonoid content of kinnow flavedo extract obtained by using different NADES combinations as solvent is shown in Fig. 4B. In ChCl:1,2 PD based NADES, highest flavonoid extraction (4.98 mg QE/g dw) was observed at 1:3 molar ratio which might be due to less viscosity that helped in better penetration of NADES inside the solid matrix as well as its less polar nature. In addition, set temperature (50 °C) contribute to decrease in viscosity which resulted in increase in extraction of flavonoid content. These results are in agreement with Xu et al. [9]who also noted DES prepared from less polar HBD such as 1,2 PD, LeA and acetamide gave highest extraction of flavonoid. The reason behind might be the basic principle of solubility i.e. ‘like dissolves like’ and citrus flavonoid (especially polymethoxy flavonoid)

present in citrus peel are less-polar in nature due to presence of numerous methoxy groups on their basic benzo-c-pyrone (15-carbon, C6-C3-C6) skeleton with a carbonyl group at the C_4 position that might have resulted in higher flavonoid extraction from 1, 2 PD based NADES due to compatible polarity nature [36]. Organic acid-based NADES showed less flavonoid content which might be due to high polar nature of these NADES as compared to alcohol-based NADES. In case of extraction from conventional solvent i.e. 80 % methanol, TFC of 4.67 mg/g dw was noted.

Antioxidant capacity of different NADES solvent

Table 3 shows the antioxidant capacity of five different solvent at molar ratios 1:1. Significant difference ($p<0.05$) as found in antioxidant capacity measured by three different methods between these five NADES solvents. Highest DPPH radical scavenging activity was observed in acid-based NADES i.e. ChCl:MA (47.91 ± 1.43 %) and ChCl:LA (40.16 ± 1.30 %) as compared to alcohol-based NADES indicating the superior hydrogen atom donating ability of organic acid-based NADES.

Table 3 Antioxidant capacity of NADES (at molar ratio 1:1)

NADES combination	DPPH (%)	ABTS (%)	FRAP (mg/l)
ChCl:1,2 PD (1:1)	7.32 ± 0.23^{e}	13.53 ± 0.36^{a}	ND*
ChCl:Gly (1:1)	15.68 ± 0.08^{c}	3.38 ± 0.12^{c}	2.98 ± 0.10^{a}
ChCl:1,3 BD (1:1)	8.19 ± 0.12^{d}	10.88 ± 0.06^{b}	ND*
ChCl:MA (1:1)	47.91 ± 1.43^{a}	2.35 ± 0.03^{d}	2.21 ± 0.06^{b}
ChCl:LA (1:1)	40.16 ± 1.30^{b}	0.59 ± 0.02^{e}	1.92 ± 0.03^{c}

Different lowercase letters in the same column indicate significant differences ($p < 0.05$)
*ND – not detected

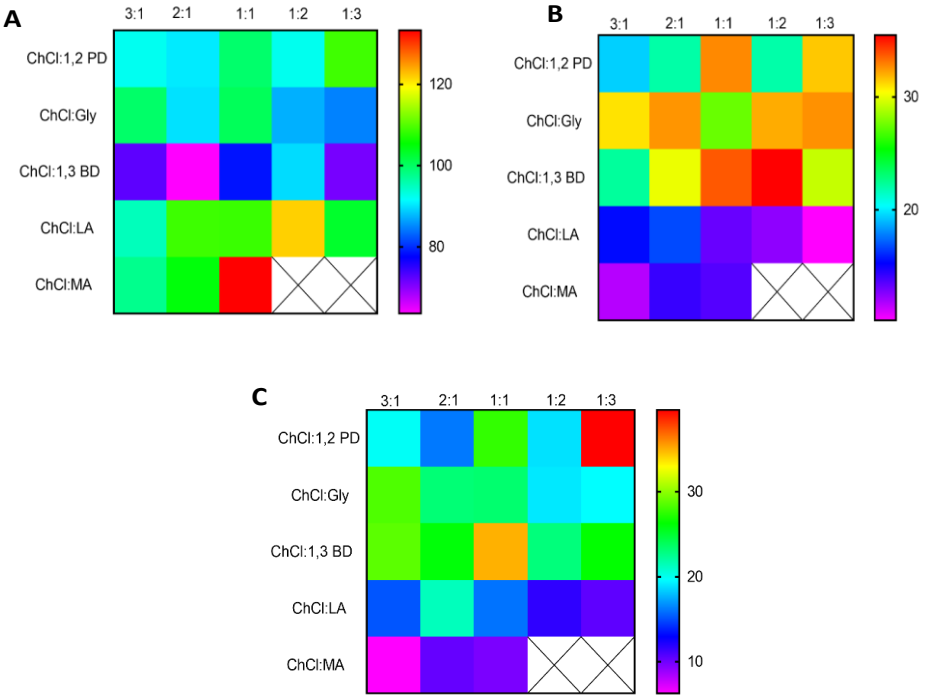
On contrary, highest radical scavenging was found in alcohol-based NADES i.e. ChCl:1,2 PD (13.53 ± 0.36 %) followed by ChCl:1,3 BD (10.88 ± 0.06) and ChCl:Gly (3.38 ± 0.12) in case of ABTS assay. In ferric reducing antioxidant power test, highest antioxidant

capacity was noted in case of ChCl:Gly whereas no antioxidant capacity was detected in ChCl:1,2 PD and ChCl:1,3 BD based NADES. Similar to our findings, [NO_PRINTED_FORM] [18]also observed good free radical scavenging property of organic acid-based NADES as compared to alcohol and sugar-based NADES.

Antioxidant capacity of Kinnow flavedo extract

Antioxidant assay generally works by two different modes of action i.e. hydrogen atom transfer (HAT) and single electron transfer (SET). DPPH and ABTS works on mixed mode (HAT and SET) whereas FRAP works on SET mechanism[37]. Therefore, in the present study, we have selected three different methods for measuring antioxidant capacity as it is always preferably to check antioxidant capacity of sample by more than one method.

Fig. 5A showed that the value of DPPH was ranging from 71.01 to 133.23 mg/g dw among different NADES combination and their molar ratios used in this study. Among all the NADES combinations with their molar ratios, highest DPPH activity was noted in the extract obtained from ChCl:MA (at molar ratio 1:1). It could be possible due to high phenol content obtained from this combination as well as free radical scavenging activity of solvent itself i.e. ChCl:MA NADES. On broader view, it can be observed that organic acid-based NADES extract (ChCl: MA and ChCl: LA) showed higher antioxidant capacity than alcohol-based ones (ChCl: 1,2 PD, ChCl: Gly and ChCl: 1,3 BD). Xu et al. [38] also found high antioxidant activity in extract obtained from organic acid based DESs than alcohol-based ones while employing extraction of bioactive compounds from *Baphicacanthus cusia* leaves. It can be inferred that the antioxidant activity of the extracts came entirely from the polyphenolic compounds of citrus flavedo in case of ChCl:1,2 PD and ChCl:1,3 BD NADES. In conventional reflux extraction, antioxidant capacity was observed 63.53 ± 8.09 mg BHT/g dw.



410

411

412 **Fig. 5** Antioxidant capacity of five NADESs at different molar ratios (A) DPPH assay (B) ABTS assay
413 (C) FRAP assay

414

415 On contrary, it can be observed that alcohol-based NADES showed higher antioxidant
416 capacity by ABTS test as compared to organic acid-based NADES (Fig. 5B). It might be due
417 to high ABTS free radical scavenging property of alcohol-based NADES as compared to
418 organic based NADES. In conventional reflux extraction, antioxidant capacity was observed
419 to be 32.10 ± 1.78 mg/g dw. Similar scenario was recorded in case of FRAP assay (Fig. 5C).
420 In conventional reflux extraction, antioxidant capacity (FRAP assay) was observed 46.61 ±
421 0.84 mg/g dw which was higher than all NADES combination.

422

423 APC Index gives a single value to antioxidant capacity measure by different methods
424 and makes easy to interpret the result. As shown in Table 4, the average of APCI index was

ranging between 39.86 to 85.45 and based on that, different ranking had been assigned. Considering the ranking, top five solvent having highest antioxidant capacity exhibiting kinnow flavedo extracts were ChCl:1,2 PD (molar ratio - 1:3) followed by 80 % methanol, ChCl:1,2 PD (molar ratio - 1:1), ChCl: 1,3 BD (molar ratio - 1:1) and ChCl:1,2 PD (molar ratio - 3:1).

Table 4 Antioxidant potency composite index (APCI) of kinnow flavedo extract

S.No.	NADES combination	Molar Ratio	DPPH index	ABTS index	FRAP index	Average of DPPH, ABTS and FRAP	Ranking
		3:1	71.50	53.49	42.52	55.84	16
		2:1	70.08	60.82	34.61	55.17	17
1.	ChCl:1,2 PD	1:1	77.22	90.31	59.11	75.55	3
		1:2	71.64	60.69	40.45	57.59	15
		1:3	84.41	86.89	85.04	85.45	1
		3:1	77.35	85.40	60.73	74.49	5
		2:1	69.73	89.76	49.75	69.75	8
2.	ChCl: Gly	1:1	78.00	75.63	56.32	69.98	7
		1:2	67.62	88.53	40.79	65.65	10
		1:3	65.65	89.86	42.07	65.86	9
		3:1	56.16	61.46	63.90	60.51	13
		2:1	49.37	82.86	55.92	62.71	12
3.	ChCl:1,3 BD	1:1	60.92	93.03	71.71	75.22	4
		1:2	69.48	97.82	49.48	72.26	6
		1:3	55.05	80.75	56.43	64.08	11
		3:1	75.80	30.31	13.45	39.86	24
4.	ChCl: MA	2:1	81.36	37.64	22.05	47.02	22
		1:1	98.39	38.38	23.26	53.34	18
		3:1	73.83	42.60	32.52	49.65	21
		2:1	84.35	46.12	45.85	58.77	14
5.	ChCl: LA	1:1	84.19	36.42	34.25	51.62	19
		1:2	94.56	34.26	25.09	51.30	20
		1:3	79.88	28.19	22.53	43.54	23
6.	80 % methanol	-	49.29	88.59	100.00	79.29	2

It can be noted from the table that top 13 ranking antioxidant capacity kinnow flavedo extract belong to alcohol-based NADES. Therefore, it can be concluded that alcohol-based NADES has greater ability to enhance the antioxidant capacity of the extract.

Cytotoxicity of NADES

Eutectic mixture is generally considered non-toxic in nature by assuming that the starting components used for the formation of NADES are natural compounds. However, this presumption should not be made without investigation of the toxicity of prepared NADES. Also, to add NADES extracts in food products, the study of toxicity is important in assessing the safety of NADES for human consumption. For assessing the potential biological activity of new compounds and verifying their toxicity, cell lines provide a useful biological system [39]. Therefore, the cytotoxicity of five best NADES combinations with the selected molar ratios (i.e. ChCl: 1,2 PD (1:3), ChCl:Gly (3:1), ChCl: 1,3 BD (1:1), ChCl: MA (1:1), and ChCl: LA (2:1)), each from different combination of solvent, on the basis of high APCI was tested on mouse splenic lymphocytes (Fig. 6). After reviewing the literature, it came in limelight that NADES combinations that contain acids as hydrogen bond donor possess higher cytotoxicity effect on cell lines. Keeping this in mind, in present study, concentration range of acid based NADES (i.e. ChCl:MA (1:1) and ChCl:LA (2:1)) were varied i.e. 0.125 to 2.0 % and tested on cell line whereas those of alcohol based ones (i.e. ChCl: 1,2 PD (1:3), ChCl:Gly (3:1) and ChCl: 1,3 BD (1:1)) were tested at higher concentration (0.93 to 15 %). Figure 3 depicts that ChCl:1,2 PD (1:3) and ChCl:Gly (3:1) were non-toxic even at higher concentration of NADES and showed cell proliferation even at 15 % concentration. Jesus et al. [40] treated L929 and HacaT cells with different NADES prepared from trehalose, proline, glucose and sorbitol and found high cell viability as well as improved cell proliferation that showed NADES do not affect the activity of cell.

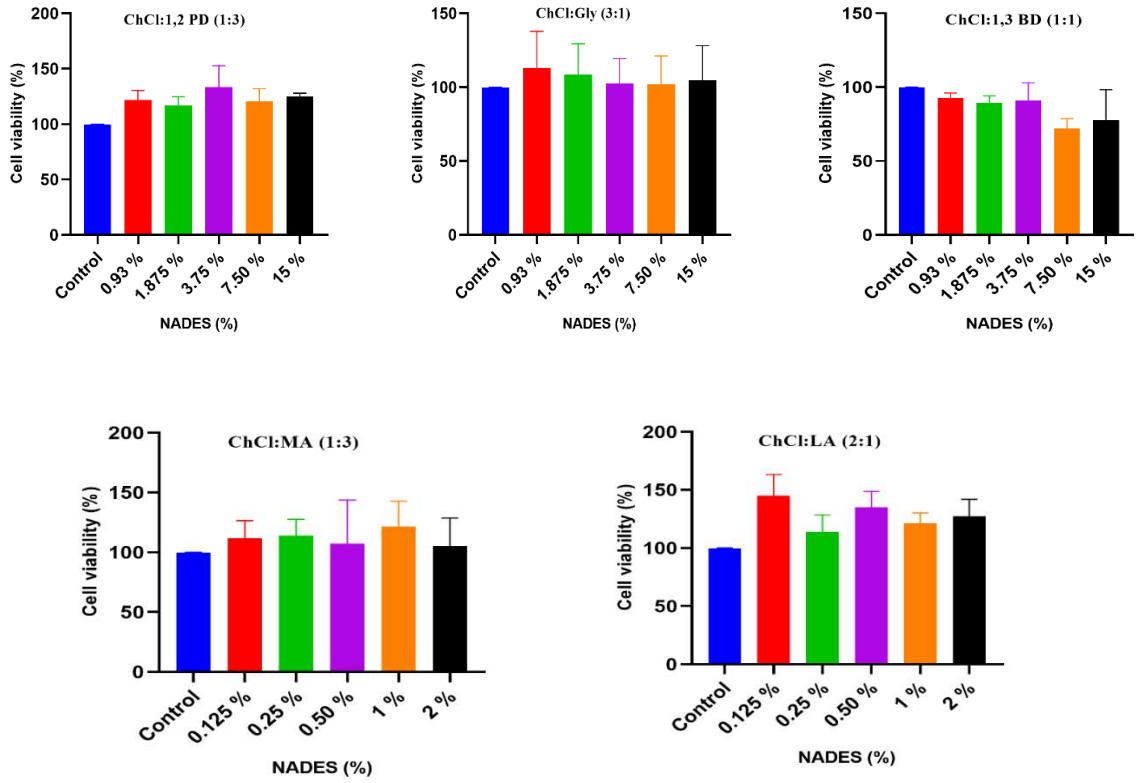


Fig. 6 Cell survival (%) after treating with alcohol and acid-based NADES on mouse splenic lymphocytes cell line

Cytotoxicity was found in case of ChCl:1,3 BD (1:1) NADES that varied from 7.52 % to 27.90 % (that means cell viability ranged from 72.10 % to 92.48 %). Based on ISO 10993-5 guidelines, cell viability values exceeding 80 % indicate non-cytotoxicity, whereas values of 60–80 %, 40–60 %, and below 40 % correspond to weak, moderate, and strong cytotoxic effects, respectively [41]. Keeping this in view, it can be concluded that ChCl:1,3 BD (1:1) showed no toxicity when concentration was 0.93 % (cell viability 92.48 %), 1.875 % (cell viability 89.17 %) and 3.75 % (cell viability 91.03 %) whereas, weak toxicity was noted at 7.50 (cell viability 72.10 %) and 15 % (cell viability 77.86 %) concentration. In ChCl:MA (1:3) and ChCl: LA (2:1) based NADES, no toxicity was observed even at higher concentration i.e. 2 %.

Conclusion

In present study, sixteen NADES combinations at their different molar ratios were analyzed for physico-chemical characteristics and extraction of bioactives along with the application of

ultrasonication. Organic acid based NADESs were observed higher in polarity than alcohol based NADESs. Findings of the study showed that higher TPC and TFC were found in ChCl:MA NADES (molar ratio 1:1) and ChCl:1,2 PD (1:3), respectively. Furthermore, on the basis of overall antioxidant potency composite index and its associated ranking, extract obtained by using ChCl: 1,2 propanediol NADES as a solvent got ranked first and selected for further study. Also, all NADES pairs were found non-toxic in nature except ChCl: 1,3 BD (1:1) at 7.50 % and 15 % of NADES. This study showed that using eco-benign NADES along with ultrasonication can be successfully practiced for extraction of bioactive compounds from kinnow flavedo and extract thus prepared (being considered for GRAS status), can be directly utilized in food industry for incorporation in various food products.

Acknowledgements

The authors acknowledge GBPUAT, Pantnagar, for providing institutional facilities and support to carry out this research. Also, first author expresses sincere gratitude to Dr. Ayon Tarafdar, Scientist, ICAR-Indian Veterinary Research Institute (IVRI), for his valuable suggestions, constructive discussions, and support in addressing experimental challenges encountered during this research work.

Author contributions

TA-Investigation, Visualization, Writing-original draft, Formal analysis; AK-Methodology; Resources; Supervision, Conceptualization, Writing-review & editing; SK-Validation, Resources; Supervision; Conceptualization, AD- Supervision; Conceptualization, NC-Supervision, Validation, OP- Supervision, Validation, AD- Supervision, PCB – Supervision, Investigation

Funding

No funding was received for present study.

Data availability

Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and Consent to participate

The study was approved by the institutional animal ethics committee (IAEC) of NIFTEM-Kundli, Sonipat (Approval number: 1/N/IAEC/2022-23/01/05). Mice used in the study were as per guidelines of the Committee for the Purpose Control and Supervision of Experiments on Animals (CCSEA), Department of Animal Husbandry and Dairying (DAHD), within the Ministry of Fisheries, Animal Husbandry and Dairying, Government of India. Mice were sacrificed by CO_2 asphyxiation in a chamber meant for the purpose. Spleen tissues were collected for the isolation of splenocytes as per the approval of a protocol given by institutional animal ethics committee (IAEC) of NIFTEM-K. The Kinnow fruits used in the present study were commercially purchased from a local market and obtained in accordance with applicable local and national guidelines.

Consent for publication

Not applicable

Competing interests

The authors declare no competing interests.

References

1. Kaur S, Panesar PS, Chopra HK (2023) Citrus processing by-products: an overlooked repository of bioactive compounds. Crit Rev Food Sci Nutr 63:67–86. https://doi.org/10.1080/10408398.2021.1943647
2. United States Department of Agriculture (2026) Citrus: World Markets and Trade
3. Ramful D, Bahorun T, Bourdon E, et al (2010) Bioactive phenolics and antioxidant propensity of flavedo extracts of Mauritian citrus fruits: Potential prophylactic ingredients for functional foods application. Toxicology 278:75–87. https://doi.org/https://doi.org/10.1016/j.tox.2010.01.012

4. Thilakarathna RCN, Siow LF, Tang T-K, Lee YY (2023) A review on application of ultrasound and ultrasound assisted technology for seed oil extraction. J Food Sci Technol 60:1222–1236. https://doi.org/10.1007/s13197-022-05359-7

5. Gómez-Urios C, Viñas-Ospino A, Puchades-Colera P, et al (2023) Choline chloride-based natural deep eutectic solvents for the extraction and stability of phenolic compounds, ascorbic acid, and antioxidant capacity from Citrus sinensis peel. LWT 177:114595. https://doi.org/https://doi.org/10.1016/j.lwt.2023.114595

6. Vo TP, Phan TH, Nguyen THP, et al (2024) Green extraction of phenolics and terpenoids from passion fruit peels using natural deep eutectic solvents. J Food Process Eng 47:e14503. https://doi.org/https://doi.org/10.1111/jfpe.14503

7. Cvjetko Bubalo M, Curko N, Tomašević M, et al (2016) Green extraction of grape skin phenolics by using deep eutectic solvents. Food Chem 200:. https://doi.org/10.1016/j.foodchem.2016.01.040

8. Viñas-Ospino A, Rita Jesus A, Paiva A, et al (2024) Comparison of green solvents for the revalorization of orange by-products: Carotenoid extraction and in vitro antioxidant activity. Food Chem 442:138530. https://doi.org/https://doi.org/10.1016/j.foodchem.2024.138530

9. Xu M, Ran L, Chen N, et al (2019) Polarity-dependent extraction of flavonoids from citrus peel waste using a tailor-made deep eutectic solvent. Food Chem 297:124970. https://doi.org/10.1016/j.foodchem.2019.124970

10. National Center for Biotechnology Information (2026) PubChem Compound Summary for CID 12699, N-Methyl-N-nitrosourea. In: National Library of Medicine

11. Ivanović M, Albreht A, Krajnc P, et al (2021) Sustainable ultrasound-assisted extraction of valuable phenolics from inflorescences of Helichrysum arenarium L. using natural deep eutectic solvents. Ind Crops Prod 160:113102. https://doi.org/10.1016/j.indcrop.2020.113102

12. Xu M, Ran L, Chen N, et al (2019) Polarity-dependent extraction of flavonoids from citrus peel waste using a tailor-made deep eutectic solvent. Food Chem 297:124970. https://doi.org/https://doi.org/10.1016/j.foodchem.2019.124970

13. Popovic BM, Micic N, Potkonjak A, et al (2022) Novel extraction of polyphenols from sour cherry pomace using natural deep eutectic solvents – Ultrafast microwave-assisted NADES preparation and extraction. Food Chem 366:130562. https://doi.org/https://doi.org/10.1016/j.foodchem.2021.130562

14. Nazir F, Nazir A, Javed S, Abid S (2021) Synthesis and Optimization of Water Based Natural Deep Eutectic Solvents and its Application as Sustainable Green Extraction Media. SSRN Electronic Journal. https://doi.org/10.2139/ssrn.3973406

15. Freitas DS, Rocha D, Castro TG, et al (2022) Green Extraction of Cork Bioactive Compounds Using Natural Deep Eutectic Mixtures. ACS Sustain Chem Eng 10:7974–7989. https://doi.org/10.1021/acssuschemeng.2c01422

16. Gómez-Urios C, Viñas-Ospino A, Puchades-Colera P, et al (2022) Sustainable Development and Storage Stability of Orange By-Products Extract Using Natural Deep Eutectic Solvents. Foods 11:2457. https://doi.org/10.3390/foods11162457

17. Boli E, Prinos N, Louli V, et al (2022) Recovery of Bioactive Extracts from Olive Leaves Using Conventional and Microwave-Assisted Extraction with Classical and Deep Eutectic Solvents. Separations 9:255. https://doi.org/10.3390/separations9090255

18. Che Zain MS, Yeoh JX, Lee SY, Shaari K (2021) Physicochemical Properties of Choline Chloride-Based Natural Deep Eutectic Solvents (NaDES) and Their Applicability for Extracting Oil Palm Flavonoids. Sustainability 13:12981. https://doi.org/10.3390/su132312981

19. Abu Bakar MF, Mohamed M, Rahmat A, Fry J (2009) Phytochemicals and antioxidant activity of different parts of bambangan (Mangifera pajang) and tarap (Artocarpus odoratissimus). Food Chem 113:479–483. https://doi.org/10.1016/j.foodchem.2008.07.081

20. Belwal T, Dhyani P, Bhatt ID, et al (2016) Optimization extraction conditions for improving phenolic content and antioxidant activity in Berberis asiatica fruits using response surface methodology (RSM). Food Chem 207:115–124. https://doi.org/10.1016/j.foodchem.2016.03.081

21. Singanusong R, Nipornram S, Tochampa W, Rattanatraiwong P (2015) Low Power Ultrasound-Assisted Extraction of Phenolic Compounds from Mandarin (Citrus reticulata Blanco cv. Sainampueng) and Lime (Citrus aurantifolia) Peels and the Antioxidant. Food Anal Methods 8:1112–1123. https://doi.org/10.1007/s12161-014-9992-6

22. Verma K, Tarafdar A, Mishra V, et al (2021) Nanoencapsulated curcumin emulsion utilizing milk cream as a potential vehicle by microfluidization: Bioaccessibility, cytotoxicity and physico-functional properties. Food Research International 148:110611. https://doi.org/10.1016/j.foodres.2021.110611

23. Ivanović M, Grujić D, Cerar J, et al (2022) Extraction of Bioactive Metabolites from Achillea millefolium L. with Choline Chloride Based Natural Deep Eutectic Solvents: A Study of the Antioxidant and Antimicrobial Activity. Antioxidants 11:724. https://doi.org/10.3390/antiox11040724

24. Cannavacciuolo C, Pagliari S, Frigerio J, et al (2022) Natural Deep Eutectic Solvents (NADESs) Combined with Sustainable Extraction Techniques: A Review of the Green Chemistry Approach in Food Analysis. Foods 12:56. https://doi.org/10.3390/foods12010056

25. Mohd Fuad F, Mohd Nadzir M, Harun@Kamaruddin A (2021) Hydrophilic natural deep eutectic solvent : A review on physicochemical properties and extractability of bioactive compounds. J Mol Liq 339:116923. https://doi.org/10.1016/j.molliq.2021.116923

26. Skulcova A, Russ A, Jablonsky M, Sima J (2018) The pH behavior of seventeen deep eutectic solvents. Bioresources 13:5042–5051. https://doi.org/10.15376/biores.13.3.5042-5051

27. Cui Q, Liu J-Z, Wang L-T, et al (2018) Sustainable deep eutectic solvents preparation and their efficiency in extraction and enrichment of main bioactive flavonoids from sea buckthorn leaves. J Clean Prod 184:826–835. https://doi.org/10.1016/j.jclepro.2018.02.295

28. Mulia K, Krisanti E, Terahadi F, Putri S (2015) Selected Natural Deep Eutectic Solvents for the Extraction of α-Mangostin from Mangosteen (Garcinia mangostana L.) Pericarp. International Journal of Technology 6:1211. https://doi.org/10.14716/ijtech.v6i7.1984

29. Finar IL (1975) Organic Chemistry, 5th Edition. Dorling Kindersley (India) Pvt. Ltd.

30. Negi T, Kumar A, Sharma SK, et al (2024) Deep eutectic solvents: Preparation, properties, and food applications. Heliyon 10:e28784. https://doi.org/10.1016/j.heliyon.2024.e28784

31. Dai Y, van Spronsen J, Witkamp G-J, et al (2013) Natural deep eutectic solvents as new potential media for green technology. Anal Chim Acta 766:61–68. https://doi.org/10.1016/j.aca.2012.12.019

32. Karadendrou M-A, Kostopoulou I, Kakokefalou V, et al (2022) L-Proline-Based Natural Deep Eutectic Solvents as Efficient Solvents and Catalysts for the Ultrasound-Assisted Synthesis of Aurones via Knoevenagel Condensation. Catalysts 12:249. https://doi.org/10.3390/catal12030249

33. Munteanu IG, Apetrei C (2021) Analytical Methods Used in Determining Antioxidant Activity: A Review. Int J Mol Sci 22:3380. https://doi.org/10.3390/ijms22073380

34. García-Roldán A, Piriou L, Jauregi P (2023) Natural deep eutectic solvents as a green extraction of polyphenols from spent coffee ground with enhanced bioactivities. Front Plant Sci 13:. https://doi.org/10.3389/fpls.2022.1072592

35. Torres-Vega J, Gómez-Alonso S, Pérez-Navarro J, Pastene-Navarrete E (2020) Green Extraction of Alkaloids and Polyphenols from Peumus boldus Leaves with Natural Deep Eutectic Solvents and Profiling by HPLC-PDA-IT-MS/MS and HPLC-QTOF-MS/MS. Plants 9:242. https://doi.org/10.3390/plants9020242

36. Liu N, Li X, Zhao P, et al (2021) A review of chemical constituents and health-promoting effects of citrus peels. Food Chem 365:130585. https://doi.org/10.1016/j.foodchem.2021.130585

37. Siddeeg A, AlKehayez NM, Abu-Hiamed HA, et al (2021) Mode of action and determination of antioxidant activity in the dietary sources: An overview. Saudi J Biol Sci 28:1633–1644. https://doi.org/10.1016/j.sjbs.2020.11.064

38. Xu Z, Cai Y, Ma Q, et al (2021) Optimization of Extraction of Bioactive Compounds from Baphicacanthus cusia Leaves by Hydrophobic Deep Eutectic Solvents. Molecules 26:1729. https://doi.org/10.3390/molecules26061729

644 39. Peng Q, Zhang Y, Zhu M, et al (2024) Polymethoxyflavones from citrus peel: advances
645 in extraction methods, biological properties, and potential applications. Crit Rev Food
646 Sci Nutr 64:5618–5630. https://doi.org/10.1080/10408398.2022.2156476

647 40. Jesus AR, Duarte ARC, Paiva A (2022) Use of natural deep eutectic systems as new
648 cryoprotectant agents in the vitrification of mammalian cells. Sci Rep 12:8095.
649 https://doi.org/10.1038/s41598-022-12365-4

650 41. López-García J, Lehocký M, Humpolíček P, Sáha P (2014) HaCaT Keratinocytes
651 Response on Antimicrobial Atelocollagen Substrates: Extent of Cytotoxicity, Cell
652 Viability and Proliferation. J Funct Biomater 5:43–57.
653 https://doi.org/10.3390/jfb5020043

654

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryfiles.docx